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INTRODUCTION:

Exsanguination is the leading cause of death on the battlefield. Lifesaving interventions include arresting hemorrhage and initiating resuscitation. The ideal resuscitation of combat casualties has not been determined. Delaying resuscitation has been shown to be beneficial in some settings and anesthetics utilized can have a profound effect on the resuscitation. The goal of this proposal is to determine the ideal resuscitation regimen of swine undergoing a Grade V liver injury followed by 30 minutes of uncontrolled hemorrhagic shock. Fluids studied include lactated Ringer's (LR), normal saline (NS) and various concentrations of hypertonic saline. Fluids were evaluated based on their effects on mortality, metabolic changes, blood pressure, tissue oxygenation and inflammatory changes measured in the lung. The effect of total intravenous anesthesia (TIVA) on the model was also studied.

BODY:

Materials and Methods

Part 1 – Auto-resuscitation in uncontrolled hemorrhagic shock

50 Yorkshire crossbred female swine, weighing a mean of 33 ±3 kg, underwent a 16-hour pre-operative fast except for water *ad libitum.* All swine were anesthetized with 8mg/kg intramuscular tiletamine hydrochloride/zolazepam hydrochloride (Telazol®, Fort Dodge Animal Health, Fort Dodge, IA), followed by oro-endotracheal intubation, mechanical ventilation, and maintenance of general anesthesia with isoflurane (Abbott Laboratories, North Chicago, IL).

Animals underwent neck cut-down and placement of 16-gauge catheters in both the carotid artery and external jugular vein for invasive blood pressure monitoring, blood sampling, and fluid administration. An InSpectra™ near-infrared, transcutaneous tissue oximeter probe (Hutchinson Technology, Hutchinson, MN) was placed on the inner right hind leg to measure tissue oxygenation (StO_2). Celiotomy was performed, as well as placement of a 16-French Foley suprapubic bladder catheter for monitoring urine output. Splenectomy was performed, and spleen replacement fluid was given using normal saline in a volume three times the mass in grams of the splenectomy specimen, followed by a 15-minute stabilization period. Continuous MAP and StO_2 measurements were recorded starting 5 minutes before the end of the stabilization period. MAP was recorded every 10 seconds using a Digi-Med BPA 400 blood pressure analyzer (Micro-Med, Inc., Louisville, KY), and StO_2 was recorded every 3 seconds using the InSpectra™ monitor.

Using a previously described technique, swine were then given a grade V liver injury with a specially designed clamp, which provided a standardized injury of one or more central hepatic veins. All swine were allowed 30 minutes of uncontrolled hemorrhage. EBL, nadir MAP, and nadir StO_2 were recorded. Blood was sampled prior to splenectomy and at the end of the 30-minute uncontrolled hemorrhage period and was analyzed for pH, base deficit, lactate, and hematocrit. Data were examined during two periods: injury-nadir and nadir-end of study.

Statistical Methods

All statistical computations were carried out using SPSS software, version 12.0 for Windows (SPSS, Inc., Chicago, IL). Means of continuous variables were compared between the time periods using a paired t-test. Correlations between continuous variables were assessed using Pearson's correlation coefficient. A p value <0.05 was used to determine statistical significance.

Part 2 – Comparison of the effects of various concentrations of hypertonic saline solutions on lung neutrophil sequestration and lung expression of IL-6, TNF-alpha and GCSF mRNA.

62 previously described swine were blindly randomized to 6 groups: control, sham, 3% HTS, 3% HTS with dextran, 7.5% HTS and 7.5 HTS with dextran. Control animals were anesthetized and immediately euthanized. Shams received two hours of general anesthesia, followed by lung harvest and euthanasia. Animals randomized to other groups received a single, 250 mL bolus of the blinded resuscitation fluid. This was followed by a 90-minute observation period, and carotid arterial blood was sampled 4 times at 30-minute intervals for clinical chemistry evaluation. Lung tissue was harvested and the animals euthanized 90 minutes after the liver injury.

Gene Expression of Pro-Inflammatory Mediators

A sample of left lung was preserved in a proprietary solution (RNAlater®; Ambion, Inc., Austin, TX) and stored at –20°C until further analysis. Total RNA was extracted from each sample and transcribed into single-strand cDNA using commercially available kits (RNeasy Mini Kit, QIAGEN, Valencia, CA; and SuperScript™ First-Strand Synthesis System, Invitrogen, Carlsbad, CA). Lung tissue expression of granulocyte colony stimulating factor (GCSF), interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF-α) was determined using Q-RT-PCR. Expression of the genes of interest was determined using TaqMan® Universal Master Mix (Applied Biosystems, Foster City, CA) and previously published sequences for forward/reverse primers and probes.[19-21] Gene expression levels were calibrated using an 18s ribosomal RNA (rRNA) endogenous control assay (Assays-On-Demand, Applied Biosystems, Foster City, CA). Q-RT-PCR assays for GCSF, IL-6, TNF-α, and 18s rRNA were performed in triplicate for all samples. Fold-increases for gene expression were calculated as the ratio of the Q-RT-PCR cycle threshold (C_T) for experimental animals to that of controls. The C_T is directly and inversely related to the number of copies of the gene of interest, such that a higher C_T reflects a lower gene copy number.

Neutrophil Sequestration Assay

The left upper lung lobes were preserved in formalin, sectioned, and stained for neutrophils using an immunohistochemical myeloperoxidase stain with hematoxylin counterstaining. Two representative sections were taken from each subject. All slides were examined by light microscopy with 400x total magnification (40x objective lens and 10x eyepiece; Leica Microsystems Inc., Bannockburn, IL). The numbers of neutrophils

in each high-powered-field (hpf) were counted, taking care to include only those neutrophils located within the alveolar walls. 5 hpf were examined per section, for a total of 10 hpf per animal.

Statistical Methods

All analyses were performed using a statistical software package for personal computers (SPSS 13.0 for Windows, SPSS, Inc., Chicago, IL). Fold-increases for GCSF, IL-6, and TNF-α were examined within each cohort, and the distribution studied for normality. Data points greater than 3 standard deviations away from the mean were excluded. Fold-increases for each gene were compared between cohorts using the Mann-Whitney U test, with significance determined by $p<0.05$. Mean numbers of lung neutrophils/hpf were compared between cohorts using the Mann-Whitney U test; significance was set at $p<0.05$.

Part 3 – Comparison of resuscitation with LR versus NS

Our group has previously shown that, using the described Grade V liver injury model, twice the volume of NS compared to LR is required to resuscitate to baseline blood pressure and maintain that blood pressure for 90 minutes after injury. We have also previously published differences in coagulation parameters in control animals resuscitated with LR and animals resuscitated with Hextend. This study was designed to compare hemodynamic and coagulation differences in animals resuscitated with LR and NS. The described liver injury model was utilized. Following 30 minutes of uncontrolled hemorrhagic shock, 20 animals were randomized to receive resuscitation with LR or NS at 165 cc/min to achieve and maintain the baseline blood pressure for 90 minutes. In addition to heart rate, blood pressure and StO2, cardiac output and systemic vascular resistance were measured. Serial coagulation parameters measured included prothrombin time, partial thromboplastin, fibrinogen levels and Thrombelastographs (Haemoscope Corp, Skokie Illinois).

Part 4 – Comparison of isoflurane and total intravenous anesthesia in uncontrolled hemorrhagic shock.

Twenty female Yorkshire crossbred swine were randomized blindly to receive either 1-3% inhaled ISO, or IV ketamine (15-33mg/kg/hr) with midazolam (1-2mg/kg/hr), and buprenorphine (0.5-10 mcg/kg/hr) for maintenance anesthesia. Animals were sedated with Telazol (4mg/kg) and induction was performed using ISO, followed by orotracheal intubation. An aural IV was placed, and randomized maintenance anesthesia was initiated and monitored by an animal technician who was independent of the study team. Depth of anesthesia was monitored in both groups using standardized criteria such as jaw laxity and painful stimuli to the nasal septum and forefoot. Animal temperature was controlled with external warming devices. Invasive lines were placed for continuous blood pressure recording and fluid resuscitation. Tissue oxygenation was measured in the left groin using near infrared spectroscopy. Celiotomy, splenectomy, and bladder catheterization were performed. After a 15-minute stabilization period, baseline mean

arterial pressure (MAP) was documented and a grade V liver injury created. This was followed by uncontrolled hemorrhage for 30 minutes. Animals were resuscitated with 8ml lactated Ringer's per ml blood loss at 165 ml/min. This volume was based on prior studies in our laboratory using the same model comparing total blood loss with total volume of fluid required to maintain the baseline MAP. The rate of infusion is half the rate administered by a Level I infuser as the animals were approximately half the weight of a normal human. MAP and tissue oxygen saturation (StO2) were continuously monitored. Laboratory data were collected every 30 minutes, and the animals were sacrificed at 120 minutes after injury.

RESULTS

Part 1

The mean total EBL was 786 ±190 mL. The mean total blood volume was 75±2 mL/kg, and the mean percentage of total blood volume lost was 32 ±8%. Mean continuous values for MAP and StO_2 are presented in Figure 1. The mean MAP and StO_2 values at various time points in the study are shown in Table 1. There was a significant decrease in MAP during the study, with an overall mean nadir of 33 ±8 mmHg ($p<0.001$ compared to mean MAP at injury). This occurred at a mean of 7:45 ±3:18 min after the liver injury. The StO_2 also significantly decreased to a mean nadir of 65 ±9% ($p<0.001$ compared to mean StO_2 at injury), which occurred at a mean of 11:28 ±9:24 min after liver injury. Pearson's correlation comparing changes in MAP and StO_2 during this period achieved statistical significance (Table 2), but the r-value was less than 0.5.

The relationships between total EBL and the changes in MAP and StO_2 were also investigated during the time from injury to nadir. There was a statistically significant inverse correlation between total EBL and nadir MAP ($r=-0.667$, $p<0.001$), as well as between total EBL and nadir StO_2 ($r=-0.419$, $p=0.002$). However, there was no significant correlation between the total EBL and the drop in MAP ($r=-0.19$, $p=0.18$). Also, the correlation between total EBL and the drop in StO_2, while statistically significant, was weak ($r=-0.285$, $p=0.045$).

During the study period from nadir to 30 minutes after injury, all animals showed a significant increase in both MAP and StO_2 without the administration of any intravenous fluid. The MAP increased to a mean of 47 ±11 mmHg ($p<0.001$), and StO_2 increased to a mean of 71 ±11 ($p<0.001$). Again, the mean changes in MAP and StO_2 showed a significant correlation (Table 2), but the r-value was less than 0.5.

Laboratory studies from baseline and 30 minutes after injury were also significantly different (Table 3). There were significant drops in base excess and hematocrit ($p<0.001$). There was also a significant drop in pH and a significant increase in lactate ($p<0.001$); however, neither was clinically significant.

Figure 1. Graph demonstrating changes in mean arterial pressure (MAP) and tissue oxygenation (StO_2) during the study period.

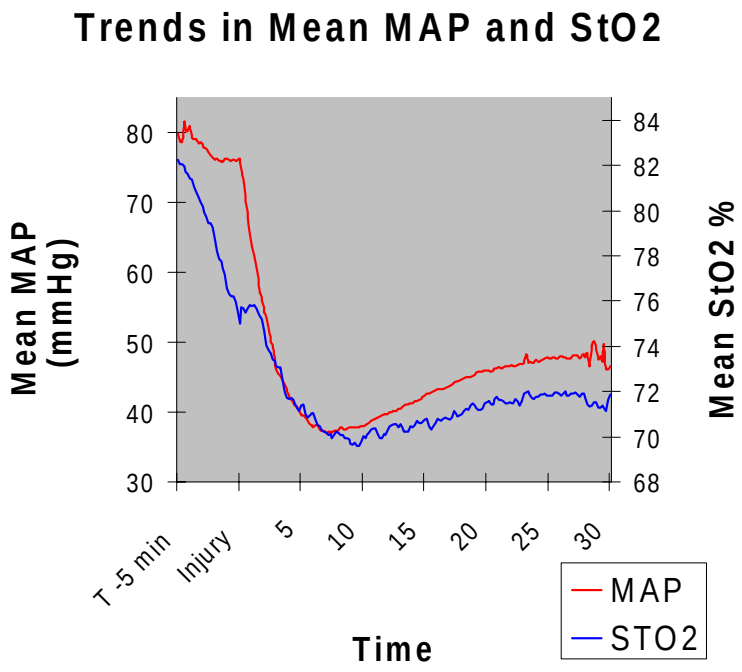


Table 1. Mean values for MAP and StO_2. The nadir values represent the mean of the lowest MAP or StO_2 for each animal during the 30 minute study period.

Study Time Point	**Mean MAP (mmHg, ±SD)**	**Mean StO_2 (%,±SD)**
Injury	76 ±17	75 ±10
Nadir	33 ±8[a]	65 ±9[a]
30 minutes	47 ±11[b]	71 ±11[b]

[a] $p<0.001$ compared to value at injury, [b] $p<0.001$ compared to value at nadir

Table 2. Mean changes in MAP and StO_2 from liver injury to nadir MAP or StO_2, and from nadir to 30 minutes after injury.

Time Interval	Mean ΔMAP (mmHg ±SD)	p	Mean ΔStO_2 (%±SD)	p	$\Delta MAP/StO_2$ Correlation (r)	p
Injury-nadir	-43 ±15	<0.001	-11 ±8	<0.001	0.42	0.002
Nadir-end study	+14 ±8	<0.001	+6 ±4	<0.001	0.35	0.012

Table 3. Laboratory data, comparing values pre-injury with those obtained at 30 minutes after injury.

Laboratory Parameter	Mean value, baseline (±SD)	Mean value, 30 min. post-injury (±SD)	p
pH	7.46 ±0.07	7.42 ±0.05	<0.001
Base excess (mEq/L)	8.2 ±3.6	4.6 ±3.4	<0.001
Lactate (mmol/L)	1.56 ±0.65	2.61 ±1.41	<0.001
Hematocrit (%)	26.1 ±2.9	22.3 ±2.6	<0.001

Part 2

All swine with liver injuries sustained similar blood pressure nadirs, patterns of liver injury, and total estimated blood losses (Table 4), and all resuscitated animals received approximately 7 mL/kg of fluid. Additionally, all subjects demonstrated a significant rise in mean arterial pressure (MAP) following administration of resuscitation fluids; 12 swine resuscitated to baseline MAP. Of the swine who achieved baseline MAP, 9 were from the cohorts receiving dextran (Table 5). The mean trend in MAP for each cohort is shown in Figure 2. Clinical chemistry data from the start and end of the experiment is presented in Table 6.

Mean fold-increases for each cohort are shown in Table 7. There was no significant difference in expression of any of the genes of interest between shams and controls. Mann-Whitney U testing showed increased GCSF expression in the cohort resuscitated with 7.5D ($p < 0.05$ compared to 3D and control, and $p = 0.01$ compared to 7.5S; Figure 3). There were no significant differences seen between other comparators.

Mean fold-increases for IL-6 expression were higher for NS, 3S, and 3D versus control animals ($p<0.05$), but no differences were seen between other comparators (Figure 4). Finally, there was decreased TNF-α expression in the 3D cohort compared to shams ($p=0.01$), but no other differences were found (Figure 5).

Mean values for neutrophil sequestration are reported in Table 8. There was significantly increased sequestration in swine resuscitated with NS, 3S, 7.5S, or 7.5D compared to shams ($p<0.01$ for all vs. shams except 7.5S vs. shams, $p=0.04$). Additionally, there was increased sequestration in swine given NS, 3S, or 7.5D compared to controls ($p=0.02$ for all vs. controls). Only the 3D cohort showed no significant difference from sham or controls. Between the resuscitated animals, there were significant increases in neutrophil sequestration in animals receiving NS vs. 7.5S ($p=0.04$) as well as 7.5D vs. 7.5S ($p=0.04$). Of the comparators which showed a difference in neutrophil sequestration, only NS versus sham and 7.5D versus 7.5S showed a concomitant difference in pro-inflammatory gene expression.

Table 4. Physiologic data (±SD). (Pre-Injury = immediately prior to injury, following stabilization period; MAP = mean arterial pressure; EBL = estimated blood loss; No. vessels injured = number of large hepatic or portal venous injuries.)

Cohort	Mean Weight (kg)	Mean Pre-Injury MAP (mmHg)	EBL (mL/kg)	Mean Nadir MAP (mmHg)	No. Vessels Injured
NS	33.3 (±2.1)	82 (±12)	24 (±7)	33 (±11)	2.0 (±0.7)
3S	33.3 (±2.1)	81 (±17)	24 (±6)	32 (±8)	2.2 (±0.6)
3D	34.7 (±3.3)	80 (±20)	23 (±5)	34 (±8)	2 (±0.9)
7.5S	34.2 (±3.4)	73 (±11)	23 (±6)	32 (±8)	2.1 (±1.0)
7.5D	33.8 (±3.6)	79 (±13)	22 (±4)	35 (±8)	2.2 (±1.5)
Sham	30.3 (±2.0)	N/A	N/A	N/A	N/A

Table 5. Swine resuscitated to baseline MAP; "% of Total" indicates the number from the cohort compared to the total number of hemorrhaged animals.

Cohort	No. Resuscitated to Baseline MAP	% of Cohort (n=10)	% of Total (n=50)
NS	0	0	0
3S	1	10	2
3D	5	50	10
7.5S	2	20	4
7.5D	4	40	8
Total	12	N/A	24

Table 6. Laboratory data pre-injury, t=0, and at end experiment, t=120 minutes (±SD).

Cohort	Na (mg/dL)		Cl (mg/dL)		Lactate (mg/dL)		pH		UNa (mg/dL)		Hct (%)	
Time (min)	0	120	0	120	0	120	0	120	0	120	0	120
NS	136 (±2)	136 (±2)	103 (±2)	107 (±3)	1.6 (±0.7)	2.6 (±2.1)	7.5 (±0.1)	7.4 (±0.1)	143 (±2)	144 (±3)	25 (±2)	24 (±3)
3S	136 (±2)	139 (±1)	103 (±3)	109 (±4)	1.2 (±0.4)	3.1 (±2.1)	7.5 (±0.1)	7.4 (±0.1)	142 (±3)	147 (±3)	26 (±3)	22 (±3)
3D	134 (±2)	140 (±1)	102 (±3)	111 (±3)	1.6 (±0.4)	2.6 (±2.3)	7.4 (±0.1)	7.4 (±0.1)	140 (±2)	146 (±3)	26 (±2)	20 (±2)
7.5S	134 (±1)	147 (±1)	101 (±2)	118 (±5)	1.5 (±0.8)	1.8 (±0.8)	7.4 (±0.02)	7.4 (±0.03)	140 (±3)	153 (±5)	26 (±1)	21 (±2)
7.5D	135 (±2)	148 (±1)	102 (±3)	121 (±4)	1.8 (±0.7)	2.1 (±1.3)	7.4 (±0.1)	7.4 (±0.1)	140 (±2)	154 (±3)	27 (±4)	17 (±3)

Table 7. Mean fold-increases in gene expression (±SD). The number of data points used per cohort is indicated as (n).

Cohort	GCSF	(n)	IL-6	(n)	TNF-α	(n)
NS	0.73 (±0.40)	(8)	8.02 (±6.72)	(8)	3.01 (±4.23)	(9)
3S	1.19 (±1.39)	(10)	5.24 (±4.05)	(9)	0.83 (±0.95)	(9)
3D	0.61 (±0.75)	(10)	5.23 (±4.65)	(9)	0.57 (±0.45)	(9)
7.5S	0.46 (±0.40)	(10)	3.17 (±3.04)	(10)	1.10 (±1.53)	(10)
7.5D	1.78 (±1.60)	(9)	25.13 (±38.99)	(9)	1.67 (±2.49)	(9)
Sham	0.97 (±1.69)	(6)	2.07 (±3.20)	(6)	1.60 (±0.41)	(4)
Control	1.00	(6)	1.00	(6)	1.00	(6)

Table 8. Mean lung neutrophil sequestration (±SD). (hpf = high-powered field)

Cohort	Mean No. Neutrophils/hpf
NS	9 (±2)[a, b]
3S	9 (±4)[a]
3D	7 (±4)
7.5S	6 (±2)[a, c]
7.5D	10 (±4)[a]
Sham	3 (±1)
Control	4 (±2)

[a] $p<0.05$ vs. sham, [b] $p<0.05$ vs. control, [c] $p<0.05$ vs. NS

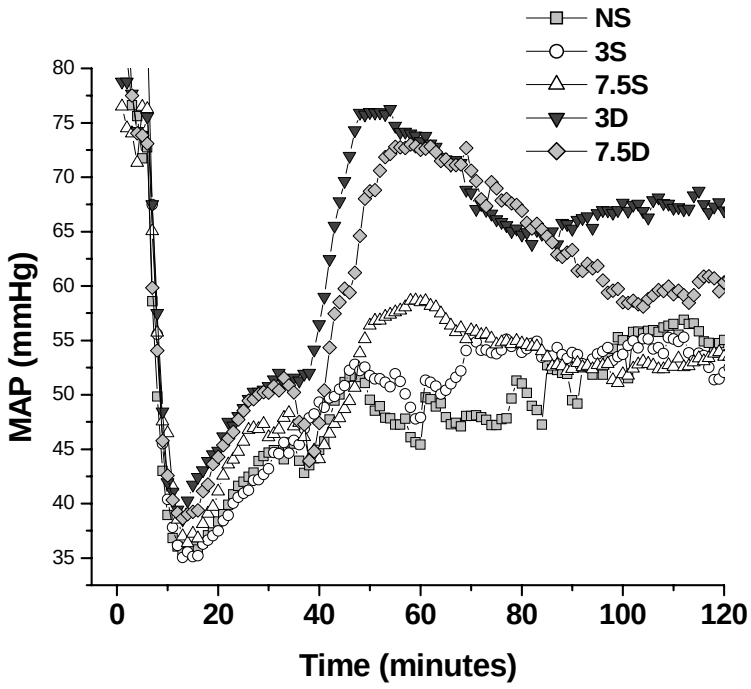


Figure 2. Mean trends in blood pressure for the resuscitation cohorts over the time-course of the experiment.

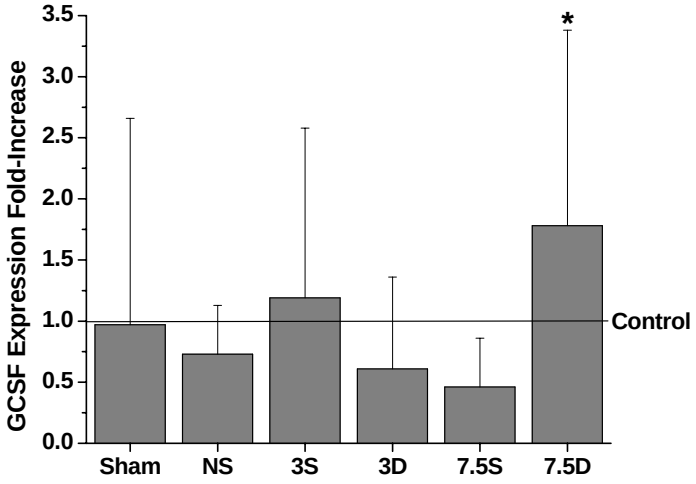


Figure 3. Fold-increases in GCSF expression by study cohort. *p<0.05 vs. 3D, 7.5S, and control.

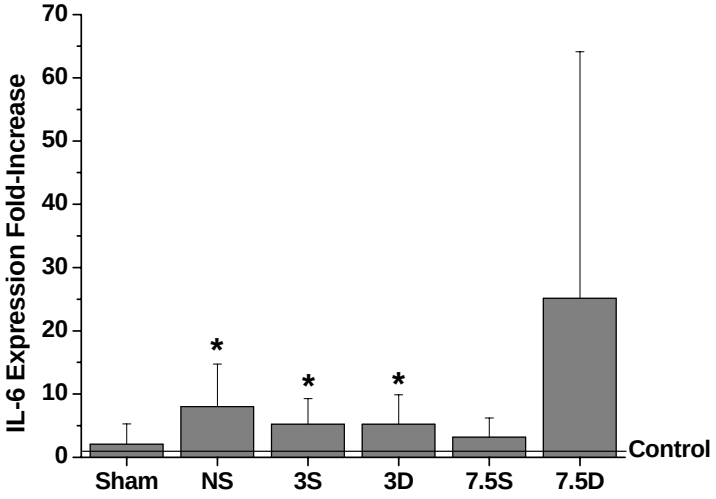


Figure 4. Fold-increases in IL-6 expression by study cohort. *p<0.05 vs. control.

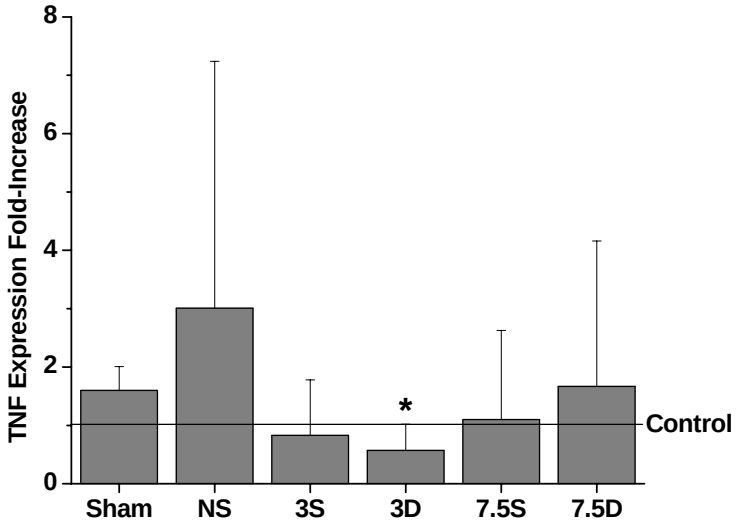


Figure 5. Fold-increase in TNF-α expression. *p<0.05 vs. control.

Part 3

Ten animals were randomized to each group. One animal in the NS group died just prior to completion of the 2 hour study period. All other animals survived. Table 9 shows the mean initial weight, blood pressure, temperature, vessels injured, blood loss and fluid replacement compared between groups. Despite the fact that the number of vessels injured and initial blood loss were similar between groups, the NS group had greater blood loss following resuscitation and required more than twice the volume of

resuscitation fluid to achieve and maintain the baseline blood pressure during the 90 minute resuscitation study period.

		Mean ± Std. Error	Statistical Significance
Survived	**NS**	9 ± .1	0.343
	LR	10 ± .0	
Weight (kg)	**NS**	33.6 ± 1.0	0.165
	LR	35.6 ± .9	
Starting Temp (C°)	**NS**	37.3 ± .6	0.356
	LR	37.9 ± .2	
Baseline MAP	**NS**	70.4±2.7	0.66
	LR	68.6±2.99	
Veins injured	**NS**	1.8±.25	0.382
	LR	1.5±.22	
Spleen replacement	**NS**	627.0 ± 52.2	0.811
fluid (cc)	**LR**	612 ± 33.2	
EBL after injury	**NS**	22.8 ± 1.9	0.102
per kg	**LR**	18.5 ± 1.7	
EBL after resuscitation	**NS**	11.6 ± 1.8	0.014 *
per kg	**LR**	5.2 ± 1.2	
Total EBL per kg	**NS**	34.3 ± 2.9	0.009 *
	LR	23.7 ± 2.1	
Fluids per kg	**NS**	330.8 ± 38.1	0.001 *
	LR	148.4 ± 20.2	

Table 9. Comparison between NS and LR groups of physiologic parameters. (* signifies statistical significance with $p < .05$)

Figure 6 compares MAP between groups during the course of the study. Although, there is a trend toward a lower blood pressure in the NS group at some time points, these differences do not reach statistical significance. Cardiac output and SVR are shown in figure 7. As the figure shows, resuscitation with NS results in a significant reduction in SVR and elevation of cardiac output. Resuscitation with NS results in decreased tissue perfusion over the course of the study.

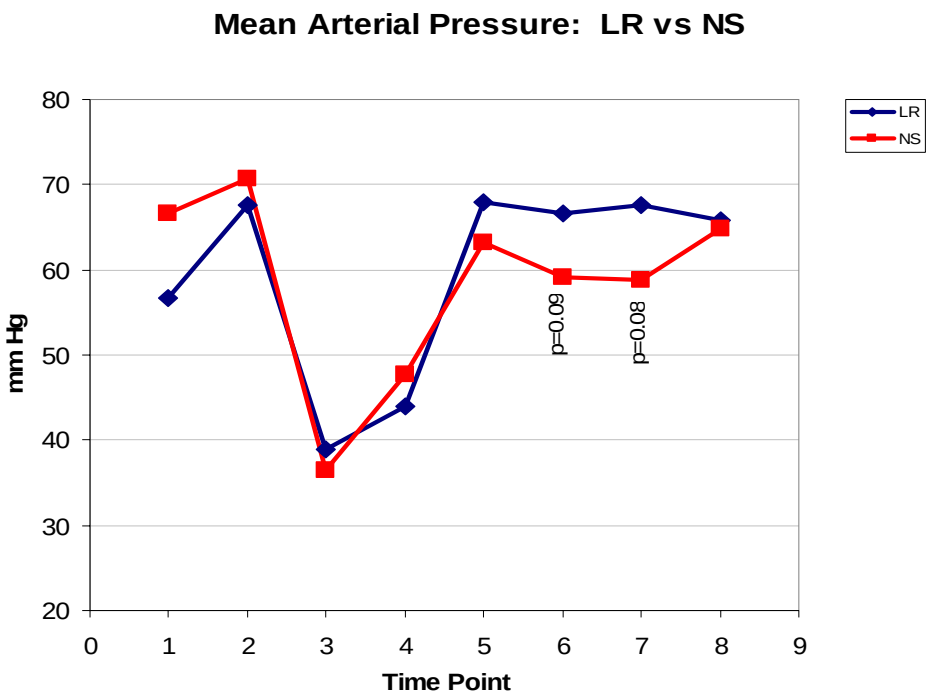


Figure 6. MAP compared between LR and NS groups.

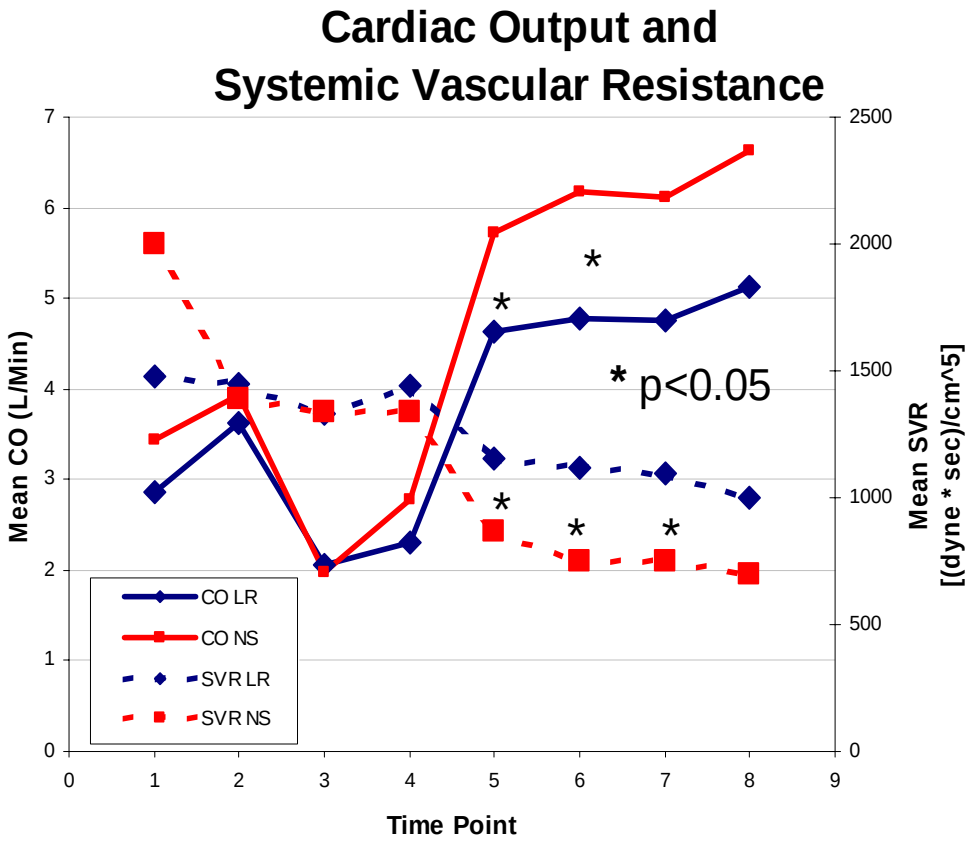


Figure 7. Comparison of CO and SVR between animals resuscitated with LR and NS.

The NS group was significantly more acidotic compared to the LR pigs after resuscitation. (Figure 8) pH was significantly lower in the NS group 30 minutes after injury until the end of study. Interestingly, at this point of the study, the only difference in treatment between the two groups was the equivalent volumes of splenic replacement fluids. The bicarbonate value and base excess were significantly lower 60 minutes after injury and beyond.

Selected laboratory values are displayed in Table 10. The two groups had equivalent hematocrit values at the start of the study. By the end of the study, the NS group had a lower hematocrit. The partial thromboplastin time (PTT) and prothrombin time (PT) were both significantly greater in the NS group compared to the LR group. Fibrinogen was decreased in both groups compared to baseline.

Figures 9-11 show the R value, alpha angle and MA of the two groups. All the parameters showed significant changes during the course of the study. At 60 minutes after injury and beyond, the R value and the alpha angle were significantly different in the LR group as compared to the NS group. At 30 minutes after injury and beyond the MA and CI were significantly higher in the LR group. By the end of the study all of the values in the groups were significantly different from baseline with the exception of the alpha angle in the NS group. These results indicate relative hypercoagulability in both groups but significantly more so in the LR group.

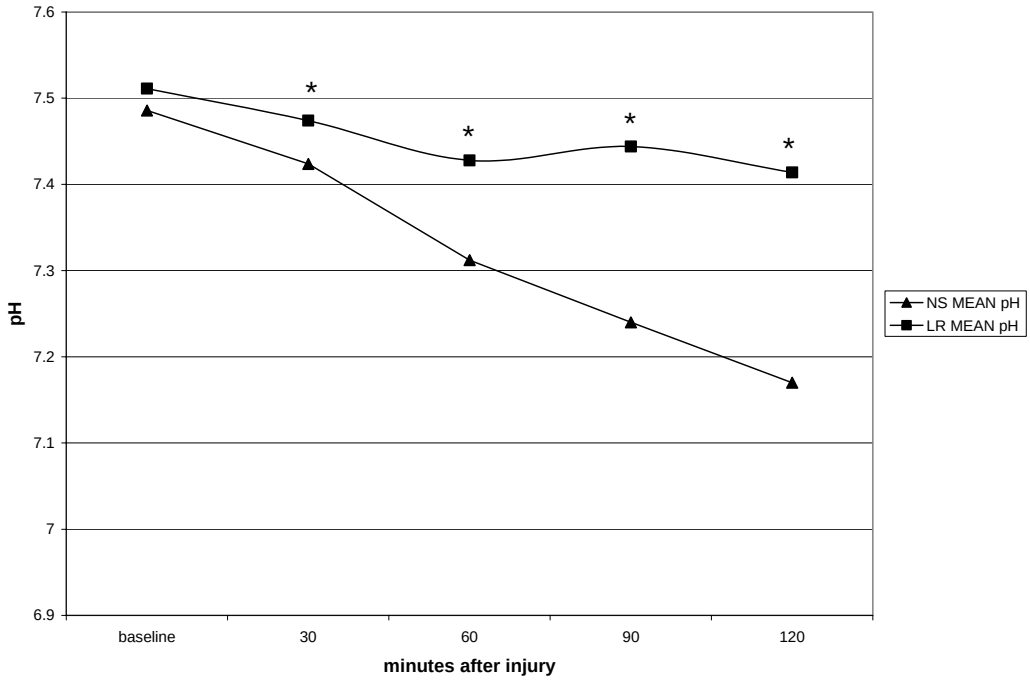
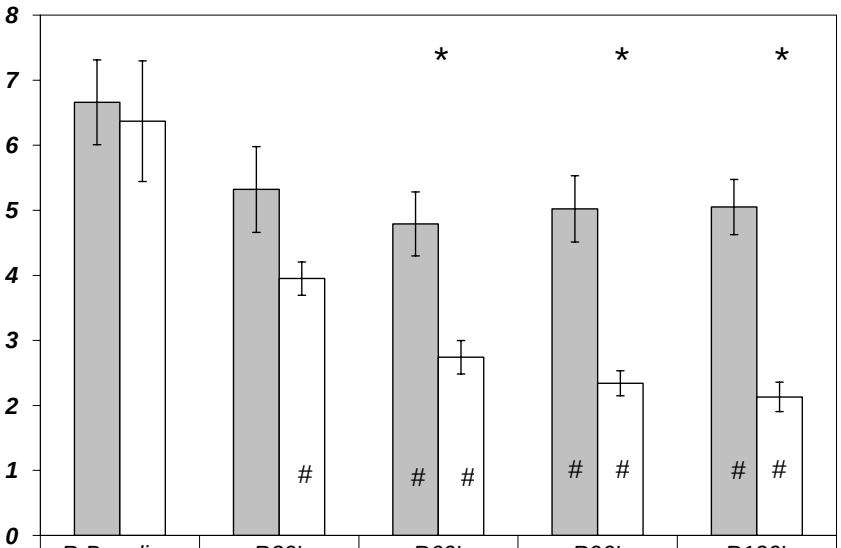


Figure 8. pH values at discrete time intervals after injury in NS and LR groups.
***** indicates a significant difference ($p < .05$) between groups at that time interval.

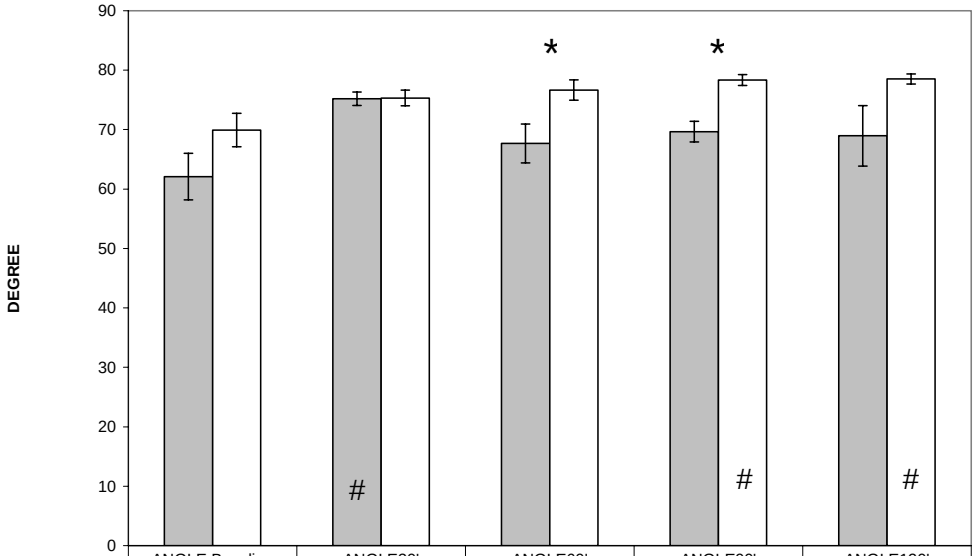
		Mean ± Std. Error	Statistical Significance
Baseline Hct	**NS**	26 ± 0.8	0.870
	LR	26.2 ± 0.9	
HCT 120 min post injury	**NS**	12.7 ± 1.1	0.028 *
	LR	16.6 ± 1.2	
Baseline PTT	**NS**	24.2 ± 1.0	0.314
	LR	22.9 ± 0.7	
PTT 120 min post injury	**NS**	25.2 ± 1.1	0.004 *
	LR	21.4 ± 0.5	
Baseline PT	**NS**	13.3 ± 0.2	0.893
	LR	13.2 ± 0.1	
PT 120 min post injury	**NS**	19.0 ± 1.5	0.037 *
	LR	15.5 ± 0.6	
Baseline Fibrinogen	**NS**	149.8 ± 12.2	0.838
	LR	146.1 ± 12.8	
Fibrinogen 120 min post injury	**NS**	68.2 ± 8.2	0.219
	LR	80.5 ± 5.5	

Table 10. Comparison between NS and LR groups of hematologic laboratory parameters drawn at discrete time points. (* signifies statistical significance with $p < .05$)



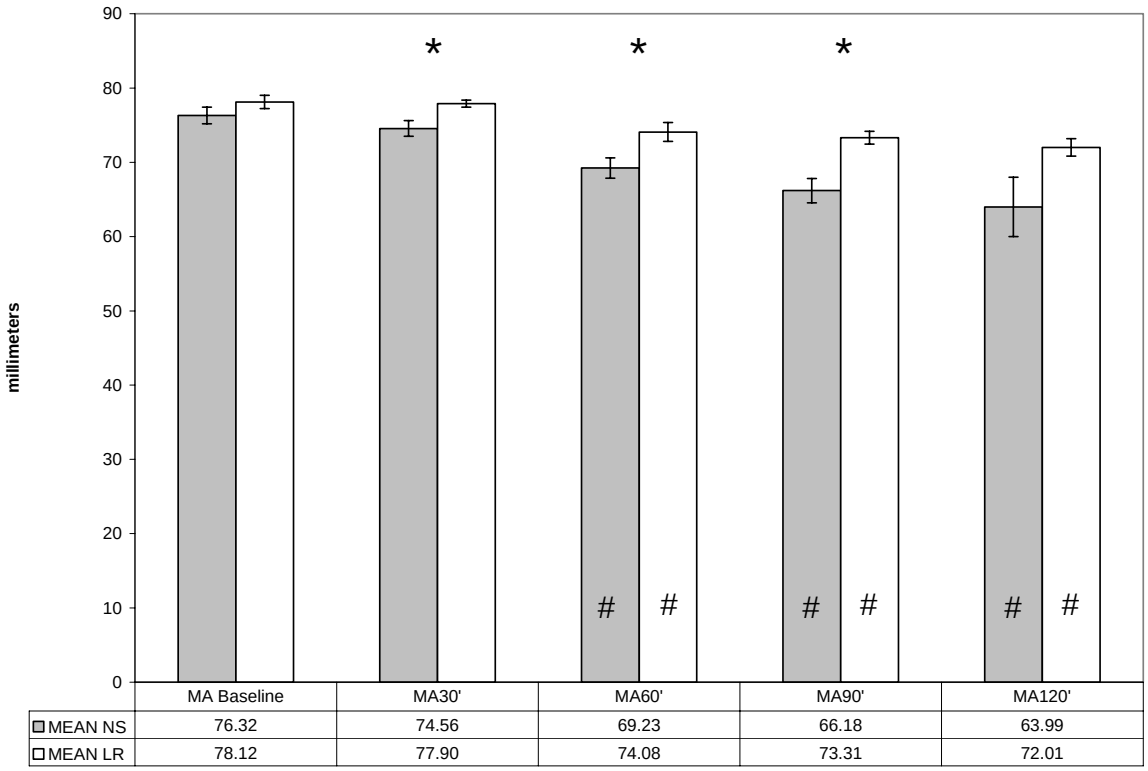
	R Baseline	R30'	R60'	R90'	R120'
MEAN NS	6.66	5.32	4.79	5.02	5.05
MEAN LR	6.37	3.95	2.74	2.34	2.13

Figure 9. TEG R values at discrete time intervals after injury in NS and LR groups. ***** indicates a significant difference ($p < .05$) between groups at that time interval. **#** indicates a significant difference from the baseline value. ($p<.05$)



	ANGLE Baseline	ANGLE30'	ANGLE60'	ANGLE90'	ANGLE120'
MEAN NS	62.1	75.19	67.67	69.65	68.95
MEAN LR	69.93	75.32	76.64	78.34	78.52

Figure 10. TEG Alpha Angle values at discrete time intervals after injury in NS and LR groups. ***** indicates a significant difference ($p < .05$) between groups at that time interval. **#** indicates a significant difference from the baseline value. ($p<.05$)



	MA Baseline	MA30'	MA60'	MA90'	MA120'
MEAN NS	76.32	74.56	69.23	66.18	63.99
MEAN LR	78.12	77.90	74.08	73.31	72.01

Figure 11. TEG MA values at discrete time intervals after injury in NS and LR groups. ***** indicates a significant difference ($p < .05$) between groups at that time interval. **#** indicates a significant difference from the baseline value. ($p<.05$)

Part 4

Based on standard criteria, all animals in both groups were adequately anesthetized using the stated ranges of anesthetics. Baseline weight (31.1 $\pm$ 0.8 kg vs. 32.5 $\pm$ 0.8 kg), number of central veins injured (1.8 for TIVA vs. 1.9 for ISO, $p>0.1$) and blood loss (796 $\pm$ 70 ml for TIVA vs. 791 $\pm$ 91 ml for ISO, $p>0.1$) were similar. Two animals in each group died prior to completing the fluid resuscitation. As shown in Figure 1, the ISO group had a lower baseline MAP (76 $\pm$ 4.0 vs. 89.5 $\pm$ 3.6, $p=0.02$), lower MAP at injury (66.6 $\pm$ 4.3 vs. 86.4 $\pm$ 4.3, $p<0.01$), and lower MAP at study completion (56.7 $\pm$ 2.7 vs. 74.9 $\pm$ 3.4, $p<0.01$). Nadir blood pressures were equivalent, and there was no difference between the two groups in the ability to return to the baseline MAP. Following resuscitation, the MAP decreased in the ISO group but stayed the same in the TIVA group. StO2 values were lower for ISO at the time of injury but were similar between the two groups for the remainder of the study (Figure 12). The acute rise in StO2 in the ISO group at 20 minutes is the result of 2 pigs dying at nearly identical time points. The ISO group had a lower lactate (3.7 $\pm$ 0.7 vs. 6.9 $\pm$ 1.1, $p=0.04$) and higher pH (7.48 $\pm$ 0.02 vs. 7.39 $\pm$ 0.03, $p= 0.04$) thirty minutes after injury but equalized following resuscitation (Figure 13).

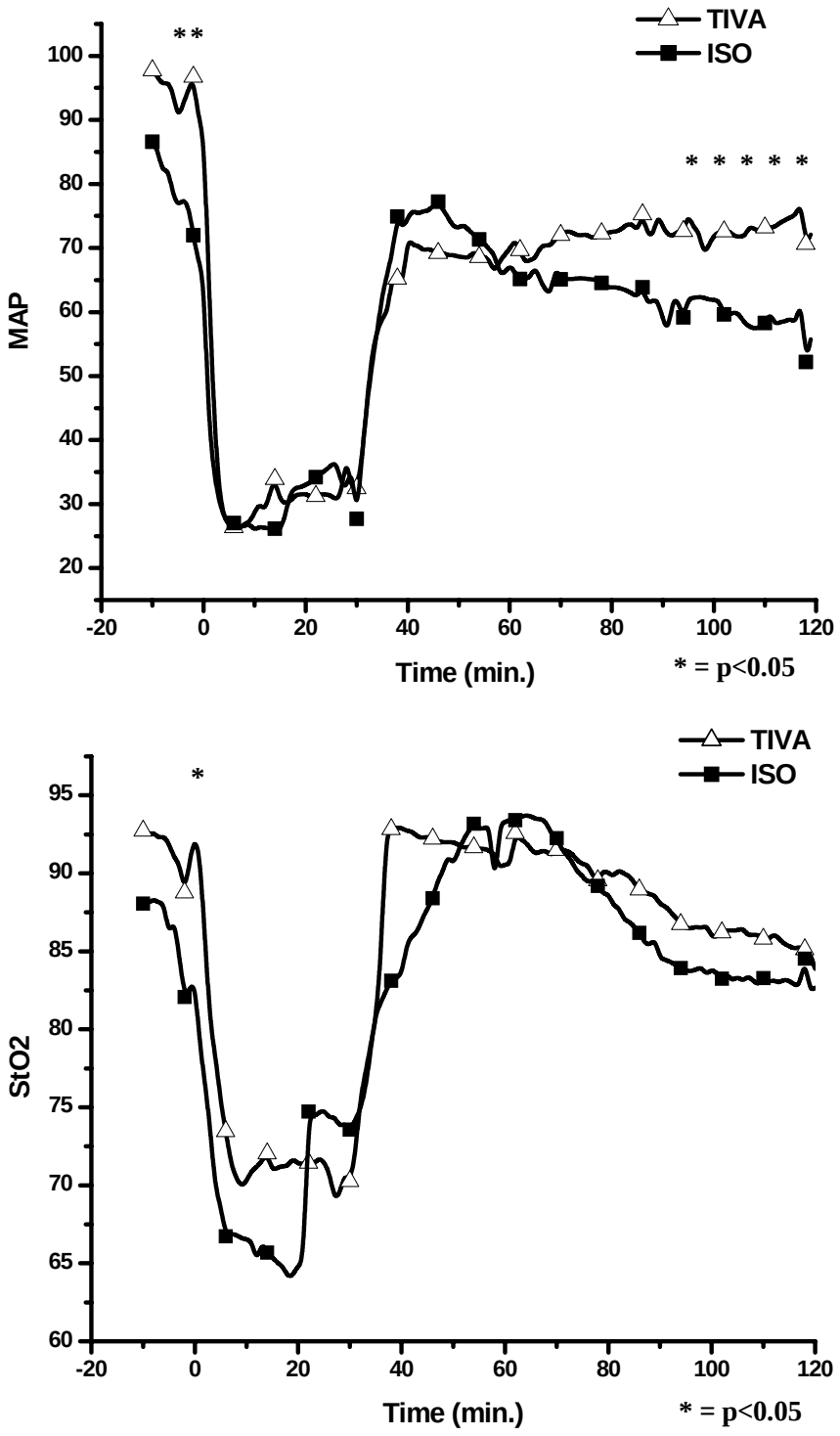


Figure 12. The effect of TIVA vs. isoflurane on MAP and StO2 in a swine model of uncontrolled hemorrhagic shock. Time point 0 represents injury.

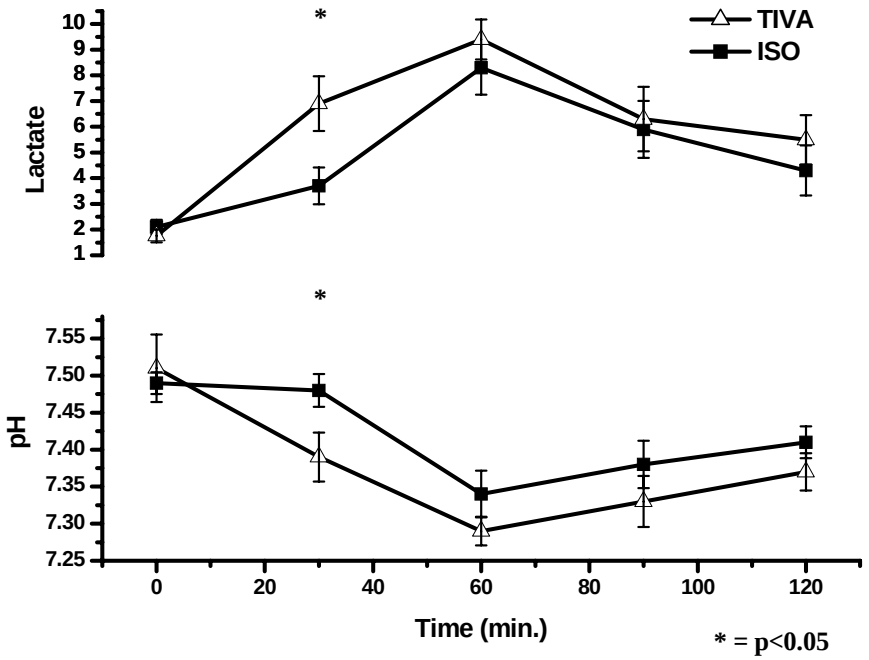


Figure 13. Comparison of pH and lactate. Time point 0 represents baseline.

KEY RESEARCH ACCOMPLISHMENTS

1. Following uncontrolled hemorrhagic shock, MAP spontaneously increases in the absence of resuscitation. This increase in MAP is associated with an increase in tissue oxygenation.
2. Change in MAP correlates with change in StO2 in uncontrolled hemorrhagic shock.
3. The addition of dextran to 7.5% hypertonic saline results in increased dysfunctional inflammation.
4. In a Grade V liver injury model of uncontrolled hemorrhagic shock, twice the volume of NS is required to resuscitate to the same blood pressure end point as LR.
5. Resuscitation of uncontrolled hemorrhagic shock with NS results in increased cardiac output and decreased systemic vascular resistance compared to LR.
6. Resuscitation of uncontrolled hemorrhagic shock with NS results in less hypercoagulability than resuscitation with LR.
7. In a swine model of uncontrolled hemorrhagic shock, total intravenous anesthesia produces adequate and comparable anesthesia to that of isoflurane.
8. Total intravenous anesthesia produces a higher MAP at baseline and results in maintenance of MAP following resuscitation without compromising tissue perfusion in uncontrolled hemorrhagic shock.

REPORTABLE OUTCOMES

Part 1 of this work was presented at the 2004 meeting of the American Association of Surgery. The abstract was published in the Journal of Surgical Research:

Differding JA, Watters JM, Muller PJ, **Schreiber MA**. The Correlation between Mean Arterial Blood Pressure and Tissue Oxygenation after Uncontrolled Hemorrhagic Shock. Journal of Surgical Research. 2004;126:336-337.

The manuscript has been submitted to the Journal of Surgical Research for publication.

Part 2 of this work was presented at the 2005 meeting of the Society of University Surgeons. The manuscript will be submitted to Shock.

Part 3 of this work was presented at the 2005 meeting of the Oregon Chapter of the American College of Surgeons and at the 2005 meeting of the Portland Surgical Society. This work is also accepted for presentation at the 2006 meeting of the Eastern Association for the Surgery of Trauma. The abstract will be published in the Journal of Trauma and the manuscript has been submitted to the Journal of Trauma.

Part 4 of this work was the winning paper at the Northwest region of the American College of Surgeons Committee on Trauma basic science resident trauma paper competition. The paper is now being considered for the national competition.